MAKING EVIDENCE-BASED GOOD AGRICULTURAL PRACTICE FOR THE REDUCTION OF MYCOTOXIN CONTAMINATION IN CEREALS

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ABSTRACT

Fusarium head blight (FHB), one of the most destructive wheat diseases in warm and humid regions, seriously threatens wheat and barley production around the world. Even worse, the mycotoxins produced by the pathogens cause food poisoning in humans and harm animals that eat infected grain. It is important for producers to realize that good agricultural practice (GAP) represents the primary line of defense against mycotoxin contamination of cereals. This paper aims to introduce the mycotoxins related to FHB and discuss evidence-based GAP for the reduction of mycotoxin contamination.

Key words: wheat, barley, deoxynivalenol (DON), nivalenol (NIV), Fusarium graminearum

INTRODUCTION

The word mycotoxin combines the Greek word “mykes,” meaning fungus, and the Latin word “toxicum,” meaning poison. Mycotoxins are present in a large part of the world’s food supply and pose a potential threat to food safety. Fusarium head blight (FHB) is a fungal disease that may infect a number of crops such as wheat, barley, oats, rye, corn, rice and forage grasses (Leonard and Bushnell 2003). However, the crops most affected in Japan are wheat and barley. FHB is not a new disease. It was recognized as a fungal infection about 120 years ago. In the last decade, there were large outbreaks of FHB in the world. Historical outbreaks of FHB can be traced to several causes: widespread planting of highly susceptible cultivars; presence of colonized residue from previous crops in the field of conservation tillage; presence of corn in rotation with small grains; and change of weather due to global warming (Leonard and Bushnell 2003). Yield losses from FHB are due to sterility of the florets and to formation of shriveled, light test-weight kernels. FHB-infected grain may be downgraded at the market. FHB not only causes yield and quality losses, but may also be associated with trichotheceen mycotoxins such as deoxynivalenol (DON) and nivalenol (NIV), and some other toxins that are hazardous to human beings and animals. In Japan, provisional guidelines for DON content in wheat grains have been provided at not more than 1.1 ppm in 2002, based on 1?ìg kg⁻¹ of body weight per day as a provisional maximum tolerable daily intake (PMTDI) for DON established by the Joint Food and Agriculture Organization-World Health Organization Expert Committee on Food Additives (JECFA) in 2001. This mycotoxin problem is the result of events at the start of the food chain. Therefore, the control of this problem calls for collaboration through the chain of production and processing.

In the meantime, we are aware of or we have played a role on good agricultural practice (GAP) in crop production. The association of the Japanese Agricultural Standard (JAS) published a manual on food safety GAP in 2005. The control practice to reduce mycotoxin contamination should be evidence-based. This paper aims to introduce a) FHB disease and its related mycotoxins; and b) our research work on evidence-based GAP for the reduction of mycotoxin contamination.
FUSARIUM HEAD BLIGHT OF CEREALS AND ITS MYCOTOXINS

Symptoms

In wheat, any part or all of the head may appear bleached (Fig. 1). These white heads are very conspicuous in a green field. Frequently, only a part of the head is affected by FHB. These partly white and partly green heads are diagnostic. Additional indications of FHB infection are pink to salmon-orange spore masses of the fungus often seen on the infected spikelet and glumes during prolonged wet weather (Fig. 1). Bleached spikelets usually are sterile or contain shriveled and/or discolored seeds (Fig. 2). These kernels are sometimes called “tombstones” because of their chalky, lifeless appearance. Other Fusarium-infected kernels may be more normal in size, if infection occurred late in kernel development. In barley, FHB infections are not always readily apparent in the field. Infected spikelets may show a browning or water-soaked appearance (Fig. 3). Infected barley kernels show a brown discoloration similar to that caused by other kernel-blighting organisms (Fig. 4).

Causal Organisms and Toxin Productivity

Several Fusarium species cause this disease, and Fusarium graminearum Schwabe [teleomorph: Gibberella zeae (Schwein.) Petch] is one of the most important species (Fig. 5) (Leonard and Bushnell 2003). F. graminearum can be divided into two chemotaxonomic groups, DON chemotype and NIV chemotype, based on the production of different trichothecene mycotoxins. DON is the most prevalent mycotoxin in cereals, and DON chemotypes of Fusarium are found worldwide. On the other hand, NIV chemotypes are found in more restricted regions; these chemotypes are found in Asia, Africa and Europe, but not in North America. Several countries have established legislative limits for DON in cereals. Thus, generally, greater attention is focused on DON than NIV as a trichothecene mycotoxin; however, NIV is also an important Fusarium mycotoxin that is frequently occurring worldwide. In Japan, where F. graminearum is the main pathogen of FHB, NIV chemotypes of F. graminearum are widely distributed and the...
isolation frequency is higher than that of DON chemotypes in some areas (Yoshida et al. 2004). Additionally, the co-occurrence of NIV and DON in wheat grain is common. Moreover, NIV is reported to be more toxic to animals than DON although the toxicological database for NIV is not as large as that for DON.

Fig. 4. Diseased seeds (right) and healthy seeds (left) in barley.

Fig. 5. Conidiospore of *Fusarium graminearum*.

**Toxicities of DON and NIV**

Trichothecen mycotoxins, especially DON and NIV (Fig. 6) possess common biochemical and cellular toxicities. These are a) the strong inhibitory effect on the protein synthesis by binding to the ribosomes; b) the inhibitory effect on RNA and DNA synthesis; and c) toxic effects on cell membrane (Sugita-Konishi and Kumagai 2005). Also, their capacity to inhibit protein synthesis is thought to induce apoptosis in thymus, lymphatic and haematopoietic tissue via mitogen-activated protein kinase. The crops contaminated with trichothecene and ingested by humans results in serious food poisoning with nausea, vomiting and diarrhea. The immunotoxicity, which is a chronic effect of trichothecene mycotoxins, results in the decrease of host resistance. Selective up regulates serum IgA caused by dietary exposure to DON or NIV induces the IgA nephropathy. The effect as a

![chemical structures](image)

Deoxynivalenol (DON)  
Nivalenol (NIV)

Fig. 6. Chemical structure of DON and NIV.
cancer promoter seems to be responsible for the immunotoxicity.

RESEARCH FOR MAKING EVIDENCE-BASED GAP

Chemical Control

The provisional standard of 1.1 ppm for DON in wheat was determined by the Japanese government in 2002. Therefore, the endpoint in our research must be changed from disease severity to mycotoxin contamination. The re-evaluation of registered fungicides and the screening of new candidates for the control of mycotoxin contamination are considered mandatory. We tested 24 kinds of fungicides with different modes of action (Table 1). Three experiments were conducted for two years (Nakajima 2004). In a paddy field, we sprayed fungicides two days before flowering and five days after flowering. Inoculations of *F. graminearum* were done at just flowering and seven days after flowering. In 2002, we used a DON producer; in 2003, we sprayed a mixture of DON and NIV producers. Sprinkler was used to promote disease development. In addition, experiment in upland field was done in 2003. Corn grain inoculum of a mixture of DON and NIV producers was used under natural rainfall condition.

As a result in 2002, most of the fungicides controlled FHB disease severity. Highly effective were tetcuconazole, captan and oxin-copper. Azoxytrobin was not so effective but efficacy was about 40. As for DON in the same test, its efficacy was lower than that of disease severity. Tetcuconazole, captan and oxin-copper decreased the DON level significantly compared with the control plot (Fig. 7-A). On the contrary, azoxystrobin increased the DON level significantly. Other fungicides did not affect the DON level.

Table 1. Fungicides tested for control of FHB and mycotoxin contamination.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Concentration</th>
<th>Mode of action</th>
<th>Registration</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLUDIOXONIL</td>
<td>1000 X</td>
<td>amino acid metabolic inhibitor</td>
<td></td>
</tr>
<tr>
<td>oxin-copper</td>
<td>400 X</td>
<td>blocking SH group</td>
<td></td>
</tr>
<tr>
<td>cooper sulfate, basic</td>
<td>4kg/10a</td>
<td>blocking SH group</td>
<td></td>
</tr>
<tr>
<td>cooper hydroxide</td>
<td>500 X</td>
<td>blocking SH group</td>
<td></td>
</tr>
<tr>
<td>thiophanate-methyl</td>
<td>4kg/10a</td>
<td>cell division inhibitor</td>
<td>wheat and barley</td>
</tr>
<tr>
<td>iminocactaide albesilate</td>
<td>1000 X</td>
<td>cell membrane and lipid</td>
<td>wheat</td>
</tr>
<tr>
<td>fenarimol</td>
<td>3000 X</td>
<td>cell membrane synthesis inhibitor</td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>1000 X</td>
<td>competition of microorganism</td>
<td>wheat and barley</td>
</tr>
<tr>
<td>sulfur</td>
<td>400 X</td>
<td>electron transfer system inhibitor</td>
<td></td>
</tr>
<tr>
<td>captan</td>
<td>600 X</td>
<td>energy metabolic inhibitor</td>
<td></td>
</tr>
<tr>
<td>isoprothiolane</td>
<td>1000 X</td>
<td>lipid metabolic inhibitor</td>
<td></td>
</tr>
<tr>
<td>probenazole</td>
<td>5000 X</td>
<td>plant activator</td>
<td></td>
</tr>
<tr>
<td>azoxystrobin + propiconazole</td>
<td>500 X</td>
<td>respiration and sterol inhibitor</td>
<td></td>
</tr>
<tr>
<td>azoxystrobin</td>
<td>2000 X</td>
<td>respiration inhibitor</td>
<td>wheat</td>
</tr>
<tr>
<td>kresoxim-methyl</td>
<td>2000 X</td>
<td>respiration inhibitor</td>
<td>wheat and barley</td>
</tr>
<tr>
<td>cyproconazole</td>
<td>3000 X</td>
<td>sterol biosynthesis inhibitor</td>
<td></td>
</tr>
<tr>
<td>myclobutanil</td>
<td>3000 X</td>
<td>sterol biosynthesis inhibitor</td>
<td></td>
</tr>
<tr>
<td>tebuconazole</td>
<td>2000 X</td>
<td>sterol biosynthesis inhibitor</td>
<td>wheat</td>
</tr>
<tr>
<td>metoconazole</td>
<td>1000 X</td>
<td>sterol biosynthesis inhibitor</td>
<td></td>
</tr>
<tr>
<td>propiconazole</td>
<td>1000 X</td>
<td>sterol biosynthesis inhibitor</td>
<td>wheat and barley</td>
</tr>
<tr>
<td>triflumizole</td>
<td>1000 X</td>
<td>sterol biosynthesis inhibitor</td>
<td>wheat and barley</td>
</tr>
<tr>
<td>hymexazol</td>
<td>500 X</td>
<td>unknown</td>
<td></td>
</tr>
<tr>
<td>phosphorous acid (4-30-16)</td>
<td>250 X</td>
<td>unknown</td>
<td></td>
</tr>
<tr>
<td>Phosphorous acid (0-28-26)</td>
<td>250 X</td>
<td>unknown</td>
<td></td>
</tr>
</tbody>
</table>
In the case of paddy field in 2003, most of the fungicides, except triflumizole, were highly effective. The reason of failure in triflumizole was unknown. As for mycotoxin in the same test, the control of DON+NIV was difficult considering the disease severity. In the condition of 2003, two-time application was not enough to decrease the mycotoxin level. Thiophanate-methyl sol, cooper hydroxide, captan and two kinds of phosphorous acid, tebuconazole and metoconazole, significantly decreased the DON+NIV level compared with the control plot (Fig. 7-B). Triflumizole was not effective in controlling both disease and toxin. Azoxystrobin and the mixture of azoxystrobin and propiconazole were effective for disease control but not for mycotoxins.

To simulate natural infection, we inoculated, in 2003, a corn grain inoculum of DON+NIV mixture in an upland field. In this case, most of the fungicides were highly effective. However, to control disease severity, a two-time application was enough in this case. As for mycotoxin in the same test, the control of DON+NIV was more difficult than that of FHB. Efficacy of toxin control was lower than that of paddy field, in which spore inoculation was done (Fig. 7-C). The corn inoculum probably supplied conidiospore continually during its maturing period. Therefore, nonvisible infection could increase the mycotoxin level. Thiophanate-methyl sol, compared with thiophanate-methyl powder, significantly decreased the DON+NIV level. Tebuconazole and metoconazole were confirmed to significantly decrease the DON+NIV level compared with the control plot. On the other hand, azoxystrobin increased the DON+NIV level, significantly the NIV level. In this case, the mixture of propiconazole and azoxystrobin neither increased or decreased the mycotoxin level. Interestingly, the mode of action of kresoxim-methyl was similar to that of azoxystrobin, but its effect on mycotoxin level seemed to be different.

Fig. 7 (A, B, C). Effects of fungicides on the level of DON and NIV: significantly different from control plot at p<0.05. Bars indicate standard error of the means. Fungicides were sprayed two days before flowering and five days after flowering (A, B, C). Inoculations were done at just flowering and seven days after flowering (A, B). In 2002, DON producer (A) was used; in 2003, a mixture of DON and NIV producers was sprayed (B). Sprinkler was used to promote disease development (A, B). The corn grain inoculum of a mixture of DON and NIV producers was used under natural rainfall condition in 2003 (C).

**CULTURAL CONTROL OF FHB AND MYCOTOXIN CONTAMINATION FOR GAP IN JAPAN**

In the United States, the introduction of non-till or minimum tillage to reduce soil erosion had led to an increasing amount of crop residue left at the soil surface, posing a high risk for FHB (Dill-Macky et al. 2000). In contrast, in Japanese wheat production, non-till or minimum tillage is not essential because of abundant rainfall and because the wheat field area is relatively small. Therefore, the cultural practice of decreasing crop residue is a practical option in Japan. Figure 8 shows some possible cultural practices to decrease the amount of crop residue. In Japan, rice straw and stub are the primary inoculum source of perithecia. Burning is an effective practice of reducing the inoculum source, however it is not environment-friendly and causes soil infertility. Japanese farmers often carry out rice straw from the paddy field for horticulture or livestock. The residue of the stub still remains in the paddy field. Another option is cultivation with the up-cut rotary, developed for clay soil-type of paddy field. This rotary reduces the amount of rice residue on the soil surface.

Soil top dressing (Fig. 9.) was a traditional cultural practice in Japan until the 1950s. Doing so was meant to recover root activity from the damage caused by frost heaving and to reduce non-available tillers. We tested a hypothesis that this cultural practice could reduce disease incidence in winter wheat (Nakajima 2004). Field experiments were conducted for four years at Morioka, northern Japan. The residues and the lower leaves infected with pink snow mold or powdery mildew were completely covered with soil at the growth stage just before leaf sheath elongation. Perithecia, the initial inoculum source of Microdochium nivale on the leaf sheath and lesion on the flag leaf, decreased.
Fig. 7 (A, B, C). Effects of fungicides on the level of DON and NIV: significantly different from control plot at p<0.05. Bars indicate standard error of the means. Fungicides were sprayed two days before flowering and five days after flowering (A, B, C). Inoculations were done at just flowering and seven days after flowering (A, B). In 2002, DON producer (A) was used; in 2003, a mixture of DON and NIV producers was sprayed (B). Sprinkler was used to promote disease development (A, B). The corn grain inoculum of a mixture of DON and NIV producers was used under natural rainfall condition in 2003 (C).
Fig. 8. Possible cultural practices to reduce FHB and mycotoxin level; burning (upper left), carry out (upper right) and up-cut rotary (lower center).

Fig. 9. Effect of soil top dressing: (right) after treatment; (left) before treatment.
by 100% and 92%, respectively. The number of diseased spikes by FSB decreased by 76%. Light was essential for perithecia formation and the survival ratio of *M. nivale* in residues decreased under the soil compared with that on soil surface. These results suggest that this cultural practice could cut the life cycle of *M. nivale*. The development of powdery mildew was delayed, increasing the weight of seeds and the total yield by 13%. On the contrary, *Rhioctonia* foot rot increased with soil top dressing (Nakajima and Naito 1998). However, in a paddy field in Kumamoto, Kyushu, soil top dressing was not so effective because of too much rice residue, a primary inoculum source of FHB.

**EFFECT OF LODGING ON THE MYCOTOXIN CONTAMINATION WITH FHB**

Lodging also seems to be an important risk factor in mycotoxin production. In Asian countries, it is common that strong winds accompanied by heavy rain near the harvest period cause lodging. A case-control study was done to clarify the effect of lodging on the accumulation of DON and NIV. Fifteen samples were collected from a naturally infested field in Japan from 2002 to 2004 (Nakajima, unpublished). Each sample was a combination of a lodged crop and non-lodged one. As shown in Fig. 10, the concentration of DON+NIV in a lodged crop was higher than that of the non-lodged one. In the case of two-row barley (Fig. 11), an increasing effect was remarkable. Additionally, trials were conducted to investigate the effect of the lodging duration. A small area of wheat inoculated with the isolate of *F. graminearum* was lodged artificially. The DON and NIV production increased by 30% even after five-seven days of lodging. These results suggest that to avoid lodging, appropriate management such as the use of an appropriate cultivar or fertilization could be a GAP for preharvest.

**CONCLUSION**

We propose the following preharvest GAPs to control *Fusarium* infection and reduce the formation of mycotoxins based on the present study and previous reference.

**Field and Soil**

Reducing the inoculum of *Fusarium* in host debris and other reservoirs in the field would be an important control measure. Consequently, reduced tillage is reported to increase the levels of deoxynivalenol in subsequent crops. Crop rotation seems to be important in reducing the inoculum, and the rotation of wheat and maize with nonhost crops of soybean or potato has been recommended. However, these evidences are weak in Japan so far and the effects have not been quantified. Basic knowledge of the distance of spore dispersal is essential to determine the area where a control practice should be done.

**Seed Treatment**

Seed treatment with fungicide and the use of quality seeds will help reduce seedling blight due to infected seeds, but will not protect against subsequent head blight. If scabby grain is to be used as a seed source, it should be thoroughly cleaned and conditioned to remove the majority of scabby kernels and to improve the test-weight. A germination test should be run to indicate germ and vigor, and seed treatment fungicides commonly used for small grains should be considered to improve the stand and vigor.

**Seeding**

Grow seed varieties developed for resistance to FHB, if available. Only seed varieties recommended for use in a particular area of a country should be planted. As far as being practical, crop planting should be timed to avoid high temperature and humidity during the period of seed development and maturation. Avoid overcrowding of plants by maintaining the recommended row and intra-plant spacing for the species/varieties grown.

**Fungicide Application**

Use of appropriate registered fungicides at the right time is the most important and effective practice for controlling FHB. We recommend that fungicide application be at the beginning of flowering, based on the fact that the plant
Fig. 10. Effect of lodging on the mycotoxin contamination with FHB for two-row barley and wheat. A significant difference between lodged and non-lodged crop was detected at $p<0.01$ according to the paired t-test.

△: two-row barley
●: wheat

Fig. 11. Effect of lodging duration on the DON level in artificially inoculated and lodged wheat field. Vertical bars indicate standard error of mean. Bars with the same letter are not significantly different at $p<0.05$ according to Tukey-Kramer’s multiple range test.
is considered to be most susceptible to infection at this stage. The frequency of fungicide application is also crucial for mycotoxin reduction. Developing control strategies which cover the late stage as well as around flowering stage would be desirable to reduce risk of DON and NIV contamination in wheat. Additional application of fungicides or other control agents at the late stage may be an effective measure, though the effective agents to reduce toxin increase at this stage could be different from those which are effective when applied at the early stage.

**Harvest**

Plan to harvest grain at low moisture content and full maturity, unless allowing the crop to continue to full maturity would subject it to hot and rainfall conditions. Delayed harvest of grain already infected by *Fusarium* species may cause a significant increase in the mycotoxin content of the crop. At harvest, the combination may be adjusted so that lightweight, FHB kernels are removed along with the chaff. However, this will not remove all FHB kernels, since some FHB infections occur late in the development of the kernel, and these infected kernels may still be fairly plump. Infected barley and oat kernels are not so easily removed in the combining process. The grain from a lodged field should not be mixed with that of a non-lodged one.

**After Harvest**

Immediate drying after harvest and proper storage prevent further contamination with DON and NIV. Physical, chemical and biological methods have been used for decontaminating grains containing trichothecene. Separation methods such as sieve sorting and gravity sorting can reduce deoxynivalenol levels in wheat. The effectiveness of milling practices for reducing the levels of DON and NIV in flour depends, to a large extent, on the degree of fungal penetration of the endosperm. Thermal processing is usually ineffective. Chemical and biological decontamination processes cannot yet be applied on a commercial scale.